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<p>(54) Title: COMBINATION OF A CHOLESTEROL BIOSYNTHESIS INHIBITOR AND A β-LACTAM CHOLESTEROL ABSORPTION INHIBITOR</p> <p>(57) Abstract</p> <p>Methods of reducing plasma cholesterol levels and treating or preventing atherosclerosis comprising administering an effective amount of a combination of a cholesterol biosynthesis inhibitor and a β-lactam cholesterol absorption inhibitor, as well as pharmaceutical compositions and kits useful in those methods, are disclosed.</p>			

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5 COMBINATION OF A CHOLESTEROL BIOSYNTHESIS
INHIBITOR AND A β -LACTAM CHOLESTEROL ABSORPTION
INHIBITOR

BACKGROUND

10 The present invention relates to a combination of a cholesterol biosynthesis inhibitor and a β -lactam cholesterol absorption inhibitor useful in reducing plasma cholesterol levels, and to a method of treating or preventing atherosclerosis comprising administering the claimed combination.

15 Plasma cholesterol levels have been positively correlated with the incidence of clinical events associated with coronary heart disease. The regulation of whole-body cholesterol homeostasis in humans and animals involves modulation of cholesterol biosynthesis, bile acid biosynthesis, and the catabolism of the cholesterol-containing

20 plasma lipoproteins. The liver is the major organ responsible for cholesterol biosynthesis and catabolism and, for this reason, it is a prime determinant of plasma cholesterol levels. The liver is the site of synthesis and secretion of very low density lipoproteins (VLDL) which are subsequently metabolized to low density lipoproteins (LDL) in the

25 circulation. LDL are the predominant cholesterol-carrying lipoproteins in the plasma and an increase in their concentration is correlated with increased atherosclerosis.

Another major factor in determining cholesterol homeostasis is the absorption of cholesterol in the small intestine. On a daily basis, the

30 average human consuming a Western diet eats 300 to 500 mg of cholesterol. In addition, 600 to 1000 mg of cholesterol can traverse the intestines each day. This latter cholesterol is a component of bile and is secreted from the liver. The process of cholesterol absorption is complex and multifaceted. It has been reported that approximately 50% of the total

35 cholesterol within the intestinal lumen is absorbed by the cells lining the intestines (*ie*, enterocytes). This cholesterol includes both diet-derived and bile- or hepatic-derived cholesterol. Much of the newly-absorbed cholesterol in the enterocytes is esterified by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT). Subsequently, these cholestry

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esters are packaged along with triglycerides and other components (*ie*, phospholipids, apoproteins) into another lipoprotein class, chylomicrons.

Chylomicrons are secreted by intestinal cells into the lymph where they can then be transported to the blood. Virtually all of the

5 cholesterol absorbed in the intestines is delivered to the liver by this route. When cholesterol absorption in the intestines is reduced, by whatever means, less cholesterol is delivered to the liver. The consequence of this action is a decreased hepatic lipoprotein (VLDL) production and an increase in the hepatic clearance of plasma cholesterol, mostly as LDL.

10 Thus, the net effect of an inhibition of intestinal cholesterol absorption is a decrease in plasma cholesterol levels.

Beta-lactams such as (3R-4S)-1,4-bis-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone disclosed in PCT/US92/05972 are potent inhibitors of intestinal cholesterol absorption, leading to decreased

15 plasma cholesterol levels in several animal species (hamsters, rats, rabbits, monkeys).

20 The inhibition of cholesterol biosynthesis by 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC1.1.1.34) inhibitors has been shown to be an effective way to reduce plasma cholesterol (Witzum, 1989) and reduce atherosclerosis. Combination therapy of an HMG CoA reductase inhibitor and a bile acid sequestrant has been demonstrated to be more effective in human hyperlipidemic patients than either agent in monotherapy (Illingworth, 1988).

25 We have unexpectedly found that a combination of a beta-lactam cholesterol absorption inhibitor and the HMG CoA reductase inhibitor lovastatin (MEVACORTM) results in a greater decrease in plasma cholesterol than either agent alone in chow-fed dogs and rhesus monkeys, and in cholesterol-fed hamsters and rabbits. These findings were unexpected because HMG CoA reductase inhibitors alone do not

30 lower plasma cholesterol levels in hamsters and monkeys.

SUMMARY OF THE INVENTION

35 The present invention relates to a method of reducing plasma cholesterol levels comprising administering to a mammal in need of such treatment an effective amount of a combination of a cholesterol biosynthesis inhibitor and a β -lactam cholesterol absorption inhibitor. The invention also relates to a method of treating or preventing atherosclerosis comprising administering an effective amount of a combination of a

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cholesterol biosynthesis inhibitor and a β -lactam cholesterol absorption inhibitor to a mammal in need of such treatment. That is, the present invention relates to the use of a β -lactam cholesterol absorption inhibitor for combined use with a cholesterol biosynthesis inhibitor (and, similarly, 5 use of a cholesterol biosynthesis inhibitor for combined use with a β -lactam cholesterol absorption inhibitor) to treat or prevent atherosclerosis or to reduce plasma cholesterol levels

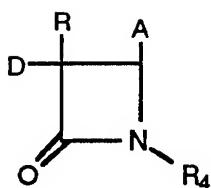
In a third aspect, the invention relates to a pharmaceutical composition comprising an effective amount of a cholesterol biosynthesis inhibitor, a β -lactam cholesterol absorption inhibitor and a 10 pharmaceutically acceptable carrier. In still another aspect, the invention relates to a kit comprising in one container an effective amount of a cholesterol biosynthesis inhibitor in a pharmaceutically acceptable carrier, and in a separate container, an effective amount of a β -lactam cholesterol absorption inhibitor in a pharmaceutically acceptable carrier. 15

DETAILED DESCRIPTION

Cholesterol biosynthesis inhibitors for use in the combination of the present invention include HMG CoA reductase inhibitors such as 20 lovastatin, pravastatin, fluvastatin, simvastatin and CI-981; HMG CoA synthetase inhibitors, for example L-659,699 ((E,E-11-[3'R-(hydroxy-methyl)-4'-oxo-2'R-oxetanyl]-3,5,7R-trimethyl-2,4-undecadienoic acid); squalene synthesis inhibitors, for example squalestatin 1; and squalene 25 epoxidase inhibitors, for example, NB-598 ((E)-N-ethyl-N-(6,6-dimethyl-2-hepten-4-ynyl)-3-[(3,3'-bithiophen-5-yl)methoxy]benzene-methanamine hydrochloride). Preferred HMG CoA reductase inhibitors are lovastatin, pravastatin and simvastatin.

β -lactam cholesterol absorption inhibitors include those 30 identified as novel compounds in formula I of PCT/US92/05972, filed July 21, 1992, and published as WO93/02048 on February 4, 1993 as well as those identified in formula II of that PCT application for use in lowering cholesterol. Said PCT application is incorporated herein by reference; formulas I and II are shown herein as follows:

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wherein

A is $-\text{CH}=\text{CH}-\text{B}$:

$$-\text{C}\equiv\text{C}-\text{B}:$$

5 -(CH₂)_p-X-B, wherein p is 0, 1 or 2 and X is a bond, -NH- or -S(O)₀₋₂₋;

heteroaryl, benzofused heteroaryl, W-substituted heteroaryl or W-substituted benzofused heteroaryl, wherein heteroaryl is selected from the group consisting of pyrrolyl, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl,

10 imidazolyl, thiazolyl, pyrazolyl, thienyl, oxazolyl and furanyl, and for nitrogen-containing heteroaryls, the N-oxides thereof, and wherein W is 1-3 substituents on the ring carbon atoms selected from the group consisting of lower alkyl, hydroxy lower alkyl, lower alkoxy, alkoxyalkyl, alkoxyalkoxy, alkoxycarbonylalkoxy, (lower alkoxy-imino)lower alkyl, lower alkanedioyl,

15 lower alkyl lower alkanedioyl, allyloxy, $-\text{CF}_3$, $-\text{OCF}_3$, benzyl, $\text{R}_{14}\text{-benzyl}$, benzyoxy, $\text{R}_{14}\text{-benzyoxy}$, phenoxy, $\text{R}_{14}\text{-phenoxy}$, dioxolanyl, NO_2 , $-\text{NR}_{10}\text{R}_{11}$, $\text{NR}_{10}\text{R}_{11}$ (lower alkyl)-, $\text{NR}_{10}\text{R}_{11}$ (lower alkoxy)-, OH, halogeno, $-\text{NHC(O)OR}_5$, $-\text{NHC(O)R}_5$, $\text{R}_6\text{O}_2\text{SNH-}$, $(\text{R}_6\text{O}_2\text{S})_2\text{N-}$, $-\text{S(O)}_2\text{NH}_2$, $-\text{S(O)}_2\text{R}_{10}$, tert-butyldimethylsilyloxy methyl, $-\text{C(O)R}_{12}$ and

20 - $\text{CH}_2 - \text{N} \begin{array}{c} \text{R}_{13} \\ | \\ \text{C}_6\text{H}_4 \end{array}$, and wherein the substituents on the substituted heteroaryl ring nitrogen atoms, when present, are selected from the group consisting of lower alkyl, lower alkoxy, $-\text{C}(\text{O})\text{OR}_5$, $-\text{C}(\text{O})\text{R}_5$, OH, $\text{NR}_{10}\text{R}_{11}$ (lower alkyl)-, $\text{NR}_{10}\text{R}_{11}$ (lower alkoxy)-, $-\text{S}(\text{O})_2\text{NH}_2$ and 2-(trimethylsilyl)ethoxymethyl;

25 -C(O)-B; or

$$-(\text{CH}_2)_k - \text{N} \begin{array}{c} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{array} \text{R}_{13} \quad \text{, wherein } k \text{ is 1 or 2:}$$

D is B'-(CH₂)_mC(O)-, wherein m is 1, 2, 3, 4 or 5;

B'-(CH₂)_q-, wherein q is 2, 3, 4, 5 or 6;

B'-(CH₂)_e-Z-(CH₂)_f, wherein Z is -O-, -C(O)-, phenylene.

30 -NR₈- or -S(O)₀₋₂-, e is 0, 1, 2, 3, 4 or 5 and r is 1, 2, 3, 4 or 5,
provided that the sum of e and r is 1, 2, 3, 4, 5 or 6;

B'-(C_2 - C_6 alkenylene)-; B'-(C_4 - C_6 alkadienylene)-;

- 5 -

B'-(CH₂)_t-Z-(C₂-C₆ alkenylene)-, wherein Z is as defined above, and wherein t is 0, 1, 2 or 3, provided that the sum of t and the number of carbon atoms in the alkenylene chain is 2, 3, 4, 5 or 6;

5 B'-(CH₂)_f-V-(CH₂)_g-, wherein V is C₃-C₆ cycloalkylene, f is 1, 2, 3, 4 or 5 and g is 0, 1, 2, 3, 4 or 5, provided that the sum of f and g is 1, 2, 3, 4, 5 or 6;

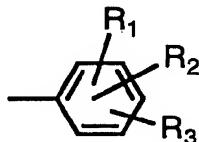
10 B'-(CH₂)_t-V-(C₂-C₆ alkenylene)- or B'-(C₂-C₆ alkenylene)-V-(CH₂)_t-, wherein V and t are as defined above, provided that the sum of t and the number of carbon atoms in the alkenylene chain is 2, 3, 4, 5 or 6;

15 B'-(CH₂)_a-Z-(CH₂)_b-V-(CH₂)_d-, wherein Z and V are as defined above and a, b and d are independently 0, 1, 2, 3, 4, 5 or 6, provided that the sum of a, b and d is 0, 1, 2, 3, 4, 5 or 6;

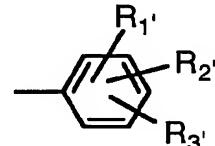
T-(CH₂)_s-, wherein T is cycloalkyl of 3-6 carbon atoms and s is 1, 2, 3, 4, 5 or 6; or

15 naphthylmethyl, heteroarylmethyl, or W-substituted heteroarylmethyl, wherein heteroaryl and W are as defined above;

B is



20 B' is naphthyl, heteroaryl or W-substituted heteroaryl, wherein heteroaryl is as defined above, or

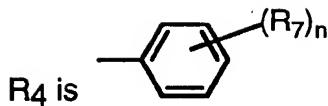


R is hydrogen, fluoro, C₁-C₁₅ alkyl, C₁-C₁₅ alkenyl, C₁-C₁₅ alkynyl, or B-(CH₂)_h-, wherein h is 0, 1, 2, or 3;

25 R₁, R₂ and R₃ are independently selected from the group consisting of H and W, provided that when W is halogeno, it is o-halogeno or m-halogeno; or R₁ is hydrogen and R₂ and R₃, together with adjacent carbon atoms to which they are attached, form a dioxolanyl ring;

30 R_{1'}, R_{2'} and R_{3'} are independently selected from the group consisting of H and W; or R_{1'} is hydrogen and R_{2'} and R_{3'}, together with adjacent carbon atoms to which they are attached, form a dioxolanyl ring;

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, wherein n is 0, 1, 2 or 3, indanyl,

benzofuranyl, benzodioxolyl, tetrahydronaphthyl, pyridyl, pyrazinyl, pyrimidinyl or quinolyl;

R₅ is lower alkyl, phenyl, R₁₄-phenyl, benzyl or R₁₄-benzyl;

5 R₆ is OH, lower alkyl, phenyl, benzyl, R₁₄-phenyl or R₁₄-benzyl;

R₇ is lower alkyl, lower alkoxy, OH, halogeno, -NR₁₀R₁₁,

-NHC(O)OR₅, -NHC(O)R₅, NO₂, -CN, -N₃, -SH, -S(O)₀₋₂-(lower alkyl),

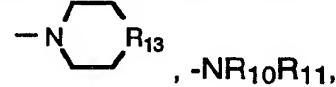
-COOR₉, -CONR₁₀R₁₁, -COR₁₂, phenoxy, benzyloxy, -OCF₃, or tert-butyldimethylsilyloxy, and where n is 2 or 3, the R₇ groups can be the

10 same or different;

R₈ is H, lower alkyl, phenyl lower alkyl, or -C(O)R₉;

R₉ is H, lower alkyl, phenyl or phenyl lower alkyl;

R₁₀ and R₁₁ are independently selected from H and lower alkyl;



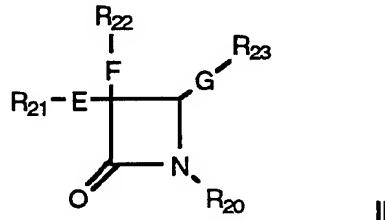
R₁₂ is H, OH, alkoxy, phenoxy, benzyloxy, -NR₁₀R₁₁,

15 lower alkyl, phenyl or R₁₄-phenyl;

R₁₃ is -O-, -CH₂-, -NH- or -N(lower alkyl)-; and

R₁₄ is 1-3 groups independently selected from the group consisting of lower alkyl, lower alkoxy, -COOH, NO₂, -NR₁₀R₁₁, OH or halogeno; or a pharmaceutically acceptable salt thereof.

20



wherein

R₂₀ is phenyl, W-substituted phenyl, naphthyl, W-substituted naphthyl, benzodioxolyl, heteroaryl, W-substituted heteroaryl, benzofused

25 heteroaryl and W-substituted benzofused heteroaryl, wherein heteroaryl is as defined above;

R₂₁, R₂₂ and R₂₃ are independently selected from H or R₂₀;

W is 1 to 3 substituents independently selected as defined above;

E, F and G are independently a bond; C₃-C₆ cycloalkylene;

30 C₁-C₁₀ alkylene; C₁-C₁₀ alkenylene; C₁-C₁₀ alkynylene; an alkylene, alkenylene or alkynylene chain as defined substituted by one or more

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substituents independently selected from the group consisting of phenyl, W-substituted phenyl, heteroaryl and W-substituted heteroaryl, wherein heteroaryl is as defined above; an alkylene, alkenylene or alkynylene chain as defined interrupted by one or more groups independently selected from the group consisting of -O-, -S-, -SO-, -SO₂-, -NR₈, -C(O)-, C₃-C₆ cycloalkylene, phenylene, W-substituted phenylene, heteroarylene and W-substituted heteroarylene; or an interrupted alkylene, alkenylene or alkynylene chain as defined substituted by one or more substituents independently selected from the group consisting of phenyl, W-substituted phenyl, heteroaryl and W-substituted heteroaryl; or one of R₂₁-E and R₂₂-F is selected from the group consisting of halogeno, OH, lower alkoxy, -OC(O)R₅, -NR₁₀R₁₁, -SH or -S(lower alkyl); and wherein R₅, R₆, and R₈-R₁₄ are as defined above; provided that when G is a bond, R₂₃ is not H, and provided that when R₂₃ is W-substituted phenyl, W is not p-halogeno; or a pharmaceutically acceptable salt thereof.

Preferred are compounds of formula I wherein R is H. Another group of preferred compounds of formula I is that wherein D is B'-(CH₂)_q-, B'-(CH₂)_e-Z-(CH₂)_r, B'-(C₂-C₆ alkenylene)-, or B'-(CH₂)_f-V-(CH₂)_g-, wherein B', Z, V, q, e, r, f, and g are as defined above. A third group of preferred compounds of formula I is that wherein R₄ is phenyl, R₇-substituted phenyl or indanyl. Still another group of preferred compounds of formula I is that wherein A is -(CH₂)_p-X-B, wherein X, B and p are as defined above.

Especially preferred are compounds of formula I wherein D is: B'-(CH₂)_q-, wherein B' is phenyl and q is 3 or 4; B'-(CH₂)_e-Z-(CH₂)_r, wherein B' is p-fluorophenyl or p-methoxyphenyl, e is zero, Z is -O-, and r is 2; B'-(C₂-C₆ alkenylene)- is 3-phenyl-1-propenyl; or B'-(CH₂)_f-V-(CH₂)_g-, wherein B' is phenyl, f is 1, V is cyclopropylene, and g is zero. Also especially preferred are compounds of formula I wherein A is -(CH₂)_p-X-B wherein p is zero and X is a bond. Preferably R₁, R₂ and R₃ in formula I are selected from H, OH, -NO₂, lower alkoxy, alkoxyalkoxy, lower alkyl lower alkandioyl, m-halogeno, NR₁₀R₁₁(lower alkoxy)-, allyloxy, phenoxy, alkoxy carbonylalkoxy and -C(O)R₁₂. Compounds of formula I wherein R₁ and R₃ are each H, and R₂ is in the para-position are more preferred.

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R₇ in formula I is preferably selected from lower alkyl, lower alkoxy, halogeno, -OCF₃, lower alkylthio, -NR₁₀R₁₁, -CN, OH, and -COR₁₂. More preferred are compounds of formula I are those wherein n is 1 and R₇ is in the para-position.

5 A preferred β -lactam cholesterol absorption inhibitor is (3R-4S)-1,4-bis-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone.

The effectiveness of the combinations of this invention for the reduction of plasma cholesterol levels is demonstrated by the following 10 test procedures. In the procedures, the β -lactam cholesterol absorption inhibitor is (3R-4S)-1,4-bis-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone (hereinafter Compound A) and the HMG CoA reductase inhibitor is lovastatin.

15 **Experiment 1 - Hypocholesterolemic effect of the combination of Compound A and lovastatin in the cholesterol-fed hamster:**
Method:

Male Golden Syrian hamsters (Charles River Labs, Wilmington, MA.) weighing between 100 and 125g were fed Wayne 20 rodent chow until study onset. At study onset (Day 1), animals were separated into groups (n=4-6/group) and fed Purina Chow #5001 supplemented with 0.5% by weight of cholesterol (Research Diets Inc., New Brunswick, NJ). Compound A at 3 mg/kg and lovastatin at 10 mg/kg were administered once daily for 7 days, starting on Day 1 via oral gavage 25 in 0.2 ml corn oil. On Day 7, animals were sacrificed by decapitation, blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA), and plasma was prepared by low speed centrifugation at 4°C.

Nonfasted plasma cholesterol levels were determined by a modification of the cholesterol oxidase method of Allain et al. (Clin. Chem., 30 20 (1974) p. 470-475), in which the reagents were available in a kit form from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Ten μ l of serum was assayed for total cholesterol in 1 ml of 0.15 M tris buffer, pH 7.0, containing p-chlorophenol (0.1%), cholesterol oxidase (0.13 U/ml), cholesterol ester hydrolase (0.13 U/ml), peroxidase (2.4 U/ml) and 4-35 aminoantipyrine (0.015%). Assays were carried out at 37°C for 10 min, along with cholesterol standards, and the absorbance of the resultant red quinone pigment was determined spectrophotometrically at 505 nm.

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Results:

Hamsters fed a 0.5% cholesterol-containing diet for 7 days showed a 2-fold increase in plasma cholesterol. The increase in plasma cholesterol is primarily in VLDL and LDL (Schnitzer- Polokoff et al, Comp. Biochem. Physiol., 99A (1991) p. 665-670). Compound A at 3 mg/kg/day resulted in a 15% reduction in plasma cholesterol levels, while lovastatin had no effect at 10 mg/kg/day (Table 1). When Compound A and lovastatin were given in combination, a reduction in plasma cholesterol levels of 31% was found, which was significantly greater than either treatment alone (Table 1).

Table 1

Group	Dose (mg/kg/day)	N	Hamster Plasma cholesterol (mg/dl)
Control	-	6	227 \pm 6
Compound A	3	4	192 \pm 6 ^a
Lovastatin	10	4	223 \pm 14
Compound A+ Lovastatin	3 10	4	156 \pm 11 ^{a,b}

Values are Means \pm SEM. ^ap<0.05 compared to control group. ^bp<0.05 compared to either Compound A alone or lovastatin alone.

15 **Experiment 2 - Hypocholesterolemic effect of Compound A in combination with lovastatin in cholesterol-fed rabbits:**

Methods:

Male New Zealand White rabbits weighing 2.4 - 2.6 kg were challenged for one week with a diet containing 1% cholesterol and 6% peanut oil. Hyper- and hypo-responding rabbits with serum cholesterol levels more than one standard deviation from the mean were excluded and four groups of rabbits with equivalent serum cholesterol levels were formed (n=8/group). The rabbits were then fed a diet containing 0. 5% cholesterol and 6% peanut oil, alone or with 0. 03% Compound A; 0.015% lovastatin; or 0.03% Compound A and 0.015% lovastatin. Non-fasting serum samples were obtained weekly for 4 weeks and serum cholesterol levels were determined as described in Experiment 1.

Results:

The one week challenge with the 1% cholesterol/6% peanut oil diet resulted in average serum cholesterol levels of approximately 1000 mg/dl (Table 2). Similar food consumption and weight gains were found among the four groups of rabbits over the 4 week study period. The dose of Compound A at 0.03% of the diet was calculated to be 14 mg/kg/day and the dose of lovastatin at 0.015% was 7 mg/kg/day. Serum cholesterol levels continued to rise in the control group from 1015 to 1358 mg/dl at the 4 week time point (Table 2). Compound A alone caused a 29% reduction in serum cholesterol at week 4 compared to week 0, while lovastatin alone caused a 33% reduction over the 4 week period, but these reductions over time were not statistically significant by ANOVA. The combination of Compound A with lovastatin caused statistically significant reductions in plasma cholesterol levels at all timepoints, with a 61% decrease at week 4 compared to week 0 (Table 2). The relative reductions in serum cholesterol levels were even greater when the 4 week values were compared to the control group, with a 47% decrease with Compound A alone, a 51% decrease with lovastatin alone, and a 72% reduction with the combined Compound A and lovastatin therapy.

20

Table 2

Group	Rabbit Serum Cholesterol Levels (mg/dl)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control	1015 ±90	1138 ±170	1316 ±164	1437 ±211	1358 ±193
Compound A (0.03% in diet)	1005 ±89	781 ±122	879 ^a ±109	808 ^a ±121	713 ^a ±112
Lovastatin (0.015% in diet)	993 ±95	895 ±150	839 ^a ±80	767 ^a ±87	667 ^a ±81
Compound A+ Lovastatin (0.03% + 0.015% in diet)	986 ±93	552 ^{a,b} ±76	506 ^{a,b} ±58	427 ^{a,b} ±62	382 ^{a,b} ±66

Values represent means±SEM with 8 rabbits/group.

^ap<0.05 compared to control group; ^bp<0.05 compared to Week 0 value by ANOVA over time for each treatment.

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Experiment 3 - Hypocholesterolemic effect of Compound A in combination with lovastatin in rhesus monkeys fed a cholesterol-free diet.

Methods:

5 Twenty rhesus monkeys (17 male, 3 female) weighing 4.4 - 8.5 kg were fed a fat-free monkey chow (Purina #5038-7) containing 5% corn oil for 2 weeks. Four groups of monkeys were formed with equivalent serum cholesterol levels and body weights (n=5/group). The monkeys were then continued on the fat-free chow containing 5% corn oil, alone or
10 with 3 mg/kg/day Compound A; 20 mg/kg/day lovastatin; or Compound A (3 mg/kg/day) and lovastatin (20 mg/kg/day). Fasting serum samples were obtained weekly for 3 weeks and serum cholesterol levels were measured as described in Experiment 1. Statistical differences were determined by ANOVA and Dunnett t tests on the change in serum cholesterol levels. A
15 probability level of p<0.05 was considered significant.

Results:

20 Control monkeys fed the fat-free chow containing 5% corn oil maintained a constant level of serum cholesterol over the three week study period (Table 3). Individually, Compound A at 3 mg/kg/day and lovastatin at 20 mg/kg/day caused slight reductions in serum cholesterol levels at 3 weeks, but these changes were not statistically significant compared to the 3-week control group. The combination of Compound A and lovastatin caused a significantly greater reduction of plasma
25 cholesterol than either treatment alone at all timepoints and reached a 25% reduction at Week 3 (Table 3).

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Table 3

Group	Rhesus Monkey Serum Cholesterol Levels (mg/dl)			
	Week 0	Week 1	Week 2	Week 3
Control	131 ±1	129 ±7	125 ±8	132 ±8
Compound A (3 mg/kg/day)	140 ±10	122 ±11	117 ±7	125 ±9
Lovastatin (20 mg/kg/day)	139 ±7	127 ±6	117 ±5	120 ±6
Compound A + Lovastatin (3 + 20 mg/kg/day)	136 ±8	108* ±7	101* ±7	102* ±8

Values represent means \pm SEM with 5 monkeys/group.

*p<0.05 compared to control group.

5 **Experiment 4 - Hypocholesterolemic effect of Compound A in combination with lovastatin in dogs fed a chow diet.**

Methods:

10 Fifteen male beagles were divided into three groups with equivalent serum cholesterol levels and body weights (n=5/group). The dogs were fed Purina Dog Chow (#5006) containing maltodextrin and either 0.0234% Compound A; or 0.0234% lovastatin; or the combination of Compound A (0.0234%) and lovastatin (0.0234%) for seven days. Serum samples were obtained at Day 0, 3 and 7, and serum total cholesterol levels were measured as described in Experiment 1.

15 Statistical differences were determined by ANOVA and a probability level of p<0.05 was considered significant.

Results:

20 Dogs fed the chow diet containing either Compound A at 0.0234% (5 mg/kg/day) or lovastatin at 0.0234% (5 mg/kg/day) resulted in serum cholesterol levels which were unchanged from baseline levels (Day 0) at Day 3 or Day 7 (Table 4). The combination of Compound A at

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5 mg/kg/day and lovastatin at 5 mg/kg/day caused a 33% reduction in serum cholesterol levels at Day 7 compared to baseline at Day 0 (Table 4). The serum cholesterol levels in the combination group were also significantly lower than levels in either group administered Compound A or lovastatin alone at Day 7. (Table 4)

Table 4

Group	Dog Serum Cholesterol Levels (mg/dl)		
	Day 0	Day 3	Day 7
Compound A (5 mg/kg/day)	114 ±5	106 ±13	109 ±10
Lovastatin (5 mg/kg/day)	107 ±10	107 ±8	114 ±9
Compound A + Lovastatin (5 mg/kg/day each)	109 ±8	89 ±4	77a,b ±3

Values represent means±SEM with 5 dogs/group.

a p<0.05 compared to Day 0.

b p<0.05 compared to Day 7 values of either Compound A alone or lovastatin alone.

10 Since the present invention relates to a method of treatment comprising the administration of a combination of two components, the components can be co-administered simultaneously or sequentially, or a single pharmaceutical composition comprising a cholesterol biosynthesis 15 inhibitor and a β -lactam cholesterol absorption inhibitor in a pharmaceutically acceptable carrier can be administered. The components of the combination can be administered individually or together in any conventional oral or parenteral dosage form such as a capsule, tablet, powder, cachet, suspension or solution. The formulations 20 can be prepared using conventional pharmaceutical excipients and additives using conventional techniques. Such pharmaceutically acceptable excipients and additives include non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, anti-oxidants, lubricants, flavorings, thickeners, coloring agents, emulsifiers and the like.

25 Representative formulations comprising a β -lactam cholesterol absorption inhibitor are disclosed in PCT/US92/05972 cited above. Representative formulations comprising a cholesterol biosynthesis inhibitor are well known in the art. It is contemplated that where the two

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active ingredients are administered as a single composition, the dosage forms as disclosed in the aforementioned PCT application may readily be modified using the knowledge of one skilled in the art.

The daily doses of the compounds in the combination of this invention for reducing plasma cholesterol levels are as follows: for cholesterol biosynthesis inhibitors, the typical dosage is 0.1 to 80 mg/kg of mammalian weight per day administered in single or divided dosages, usually once or twice a day; for the β -lactam cholesterol absorption inhibitor, the typical dosage is 0.1 to 10 mg/kg mammalian weight per day in single or divided dosages. The exact dose of any component of the combination to be administered is determined by the attending clinician and is dependent on the potency of the compound administered, the age, weight, condition and response of the patient.

Generally, to reduce the plasma cholesterol levels in mammals needing such treatment, the compounds in the combination of this invention may be administered to patients in dosage ranges as follows: for HMG CoA reductase inhibitors, about 10 to about 40 mg per dose is given 1 to 2 times a day, giving a total daily dose of about 10 to 80 mg per day, and for the other cholesterol biosynthesis inhibitors, about 1 to 1000 mg per dose is given 1 to 2 times a day, giving a total daily dose of about 1 mg to about 2 g per day. About 1 to about 1000 mg per dose of the β -lactam cholesterol absorption inhibitor is given 1 to 4 times a day. Where the components of a combination are administered separately, the number of doses of each component given per day may not necessarily be the same, e.g. where one component may have a greater duration of activity, and will therefore need to be administered less frequently.

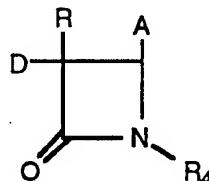
Since the present invention relates to the reduction of plasma cholesterol levels by treatment with a combination of active ingredients wherein said active ingredients may be administered separately, the invention also relates to combining separate pharmaceutical compositions in kit form. That is, a kit is contemplated wherein two separate units are combined: a cholesterol biosynthesis inhibitor pharmaceutical composition and a β -lactam cholesterol absorption inhibitor pharmaceutical composition. The kit will preferably include directions for the administration of the separate components. The kit form is particularly advantageous when the separate components must be administered in different dosage forms (e.g. oral and parenteral) or are administered at different dosage intervals.

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We claim:

1. The use of a β -lactam cholesterol absorption inhibitor for the manufacture of a medicament for the combined use with a cholesterol biosynthesis inhibitor in the treatment or prevention of atherosclerosis, or for the reduction of plasma cholesterol levels.
- 5 2. The use as claimed in claim 1, wherein the β -lactam cholesterol absorption inhibitor is represented by the structural formula

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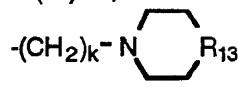


wherein

A is $-\text{CH}=\text{CH}-\text{B}$;
 $-\text{C}\equiv\text{C}-\text{B}$;
15 $-(\text{CH}_2)_p-\text{X}-\text{B}$, wherein p is 0, 1 or 2 and X is a bond, $-\text{NH}-$ or
 $-\text{S}(\text{O})_0-2-$;
heteroaryl, benzofused heteroaryl, W-substituted heteroaryl or
W-substituted benzofused heteroaryl, wherein heteroaryl is selected from
the group consisting of pyrrolyl, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl,
20 imidazolyl, thiazolyl, pyrazolyl, thienyl, oxazolyl and furanyl, and for
nitrogen-containing heteroaryls, the N-oxides thereof, and wherein W is 1-
3 substituents on the ring carbon atoms selected from the group consisting
of lower alkyl, hydroxy lower alkyl, lower alkoxy, alkoxyalkyl, alkoxyalkoxy,
alkoxycarbonylalkoxy, (lower alkoxy-imino)lower alkyl, lower alkanedioyl,
25 lower alkyl lower alkanedioyl, allyloxy, $-\text{CF}_3$, $-\text{OCF}_3$, benzyl, $\text{R}_{14}\text{-benzyl}$,
benzyloxy, $\text{R}_{14}\text{-benzyloxy}$, phenoxy, $\text{R}_{14}\text{-phenoxy}$, dioxolanyl, NO_2 ,
 $-\text{NR}_{10}\text{R}_{11}$, $\text{NR}_{10}\text{R}_{11}(\text{lower alkyl})-$, $\text{NR}_{10}\text{R}_{11}(\text{lower alkoxy})-$, OH, halogeno,
 $-\text{NHC}(\text{O})\text{OR}_5$, $-\text{NHC}(\text{O})\text{R}_5$, $\text{R}_6\text{O}_2\text{SNH}-$, $(\text{R}_6\text{O}_2\text{S})_2\text{N}-$, $-\text{S}(\text{O})_2\text{NH}_2$,
 $-\text{S}(\text{O})_0-2\text{R}_{10}$, tert-butyldimethylsilyloxymethyl, $-\text{C}(\text{O})\text{R}_{12}$ and
30 $-\text{CH}_2-\text{N}(\text{R}_{13})_2$, and wherein the substituents on the substituted
heteroaryl ring nitrogen atoms, when present, are selected from the group
consisting of lower alkyl, lower alkoxy, $-\text{C}(\text{O})\text{OR}_5$, $-\text{C}(\text{O})\text{R}_5$, OH,
 $\text{NR}_{10}\text{R}_{11}(\text{lower alkyl})-$, $\text{NR}_{10}\text{R}_{11}(\text{lower alkoxy})-$, $-\text{S}(\text{O})_2\text{NH}_2$ and
2-(trimethylsilyl)ethoxymethyl;

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-C(O)-B; or



, wherein k is 1 or 2;

D is $B'-(CH_2)_mC(O)-$, wherein m is 1, 2, 3, 4 or 5;

$B'-(CH_2)_q-$, wherein q is 2, 3, 4, 5 or 6;

5 $B'-(CH_2)_e-Z-(CH_2)_r$, wherein Z is -O-, -C(O)-, phenylene,
 $-NR_8-$ or $-S(O)_{0-2-}$, e is 0, 1, 2, 3, 4 or 5 and r is 1, 2, 3, 4 or 5,

provided that the sum of e and r is 1, 2, 3, 4, 5 or 6;

$B'-(C_2-C_6 \text{ alkenylene})-$; $B'-(C_4-C_6 \text{ alkadienylene})-$;

$B'-(CH_2)_t-Z-(C_2-C_6 \text{ alkenylene})-$, wherein Z is as defined above,

10 and wherein t is 0, 1, 2 or 3, provided that the sum of t and the number of carbon atoms in the alkenylene chain is 2, 3, 4, 5 or 6;

$B'-(CH_2)_f-V-(CH_2)_g-$, wherein V is C_3-C_6 cycloalkylene, f is 1, 2, 3, 4 or 5 and g is 0, 1, 2, 3, 4 or 5, provided that the sum of f and g is 1, 2, 3, 4, 5 or 6;

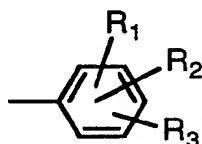
15 $B'-(CH_2)_t-V-(C_2-C_6 \text{ alkenylene})-$ or $B'-(C_2-C_6 \text{ alkenylene})-V-(CH_2)_t-$, wherein V and t are as defined above, provided that the sum of t and the number of carbon atoms in the alkenylene chain is 2, 3, 4, 5 or 6;

$B'-(CH_2)_a-Z-(CH_2)_b-V-(CH_2)_d-$, wherein Z and V are as defined above and a, b and d are independently 0, 1, 2, 3, 4, 5 or 6, provided that 20 the sum of a, b and d is 0, 1, 2, 3, 4, 5 or 6;

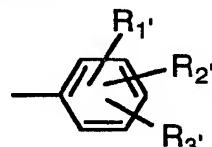
$T-(CH_2)_s-$, wherein T is cycloalkyl of 3-6 carbon atoms and s is 1, 2, 3, 4, 5 or 6; or

naphthylmethyl, heteroaryl methyl, or W-substituted heteroaryl methyl, wherein heteroaryl and W are as defined above;

25 B is



B' is naphthyl, heteroaryl or W-substituted heteroaryl, wherein heteroaryl is as defined above, or



30 R is hydrogen, fluoro, C_1-C_{15} alkyl, C_1-C_{15} alkenyl, C_1-C_{15} alkynyl, or $B-(CH_2)_h-$, wherein h is 0, 1, 2, or 3;

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R₁, R₂ and R₃ are independently selected from the group consisting of H and W, provided that when W is halogeno, it is o-halogeno or m-halogeno; or R₁ is hydrogen and R₂ and R₃, together with adjacent carbon atoms to which they are attached, form a dioxolanyl ring;

5 R_{1'}, R_{2'} and R_{3'} are independently selected from the group consisting of H and W; or R_{1'} is hydrogen and R_{2'} and R_{3'}, together with adjacent carbon atoms to which they are attached, form a dioxolanyl ring;

R₄ is  , wherein n is 0, 1, 2 or 3, indanyl, benzofuranyl, benzodioxolyl, tetrahydronaphthyl, pyridyl, pyrazinyl, 10 pyrimidinyl or quinolyl;

R₅ is lower alkyl, phenyl, R₁₄-phenyl, benzyl or R₁₄-benzyl;

R₆ is OH, lower alkyl, phenyl, benzyl, R₁₄-phenyl or R₁₄-benzyl;

R₇ is lower alkyl, lower alkoxy, OH, halogeno, -NR₁₀R₁₁,

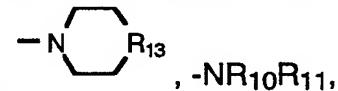
-NHC(O)OR₅, -NHC(O)R₅, NO₂, -CN, -N₃, -SH, -S(O)o-2-(lower alkyl),

15 -COOR₉, -CONR₁₀R₁₁, -COR₁₂, phenoxy, benzyloxy, -OCF₃, or tert-butyldimethylsilyloxy, and where n is 2 or 3, the R₇ groups can be the same or different;

R₈ is H, lower alkyl, phenyl lower alkyl, or -C(O)R₉;

R₉ is H, lower alkyl, phenyl or phenyl lower alkyl;

20 R₁₀ and R₁₁ are independently selected from H and lower alkyl;



R₁₂ is H, OH, alkoxy, phenoxy, benzyloxy, -NR₁₀R₁₁,

lower alkyl, phenyl or R₁₄-phenyl;

R₁₃ is -O-, -CH₂-, -NH- or -N(lower alkyl)-; and

25 R₁₄ is 1-3 groups independently selected from the group consisting of lower alkyl, lower alkoxy, -COOH, NO₂, -NR₁₀R₁₁, OH or halogeno; or a pharmaceutically acceptable salt thereof.

3. The use as claimed in claim 2, wherein the β -lactam cholesterol absorption inhibitor is as defined in claim 2, wherein:

30 R is H;

D is B'-(CH₂)_q-, B'-(CH₂)_e-Z-(CH₂)_r, B'-(C₂-C₆ alkenylene)-, or B'-(CH₂)_f-V-(CH₂)_g-, wherein B', Z, V, q, e, r, f, and g are as defined in claim 4;

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R₄ is phenyl, R₇-substituted phenyl or indanyl, wherein R₇ is lower alkyl, lower alkoxy, halogeno, -OCF₃, lower alkylthio, -NR₁₀R₁₁, -CN, OH or -COR₁₂, wherein R₁₀, R₁₁ and R₁₂ are as defined in claim 4; and A is -(CH₂)_p-X-B, wherein X, B and p are as defined in claim 4.

5

4. The use as claimed in claim 3, wherein the β -lactam cholesterol absorption inhibitor is as defined in claim 2, wherein:

D is B'-(CH₂)_q-, wherein B' is phenyl and q is 3 or 4; B'-(CH₂)_e-Z-(CH₂)_r, wherein B' is p-fluoro-phenyl or p-methoxyphenyl, e is zero, Z is 10 -O-, and r is 2; B'-(C₂-C₆ alkenylene)- is 3-phenyl-1-propenyl; or B'-(CH₂)_f-V-(CH₂)_g-, wherein B' is phenyl, f is 1, V is cyclopropylene, and g is zero;

A is -(CH₂)_p-X-B wherein p is zero, X is a bond and B is as defined in claim 4;

15

R₁, R₂ and R₃ are selected from the group consisting of H, OH, -NO₂, lower alkoxy, alkoxyalkoxy, lower alkyl lower alkandioyl, m-halogeno, NR₁₀R₁₁(lower alkoxy)-, allyloxy, phenoxy, alkoxycarbonyl-alkoxy and -C(O)R₁₂, wherein R₁₀, R₁₁ and R₁₂ are as defined in claim 4;

20

R₄ is (R₇)_n-substituted phenyl, wherein n is 1 and R₇ is lower alkyl, lower alkoxy, halogeno, -OCF₃, lower alkylthio, -NR₁₀R₁₁, -CN, OH or -COR₁₂, wherein R₁₀, R₁₁ and R₁₂ are as defined in claim 4.

25

5. The use as claimed in claim 4, wherein the β -lactam cholesterol absorption inhibitor is (3R-4S)-1,4-bis-(4-methoxy-phenyl)-3-(3-phenylpropyl)-2-azetidinone.

30

6. The use of a cholesterol biosynthesis inhibitor for the manufacture of a medicament for the combined use with a β -lactam cholesterol absorption inhibitor in the treatment or prevention of atherosclerosis, or for the reduction of plasma cholesterol levels.

35

7. The use as claimed in claim 6, wherein the cholesterol biosynthesis inhibitor is selected from the group consisting of HMG CoA reductase inhibitors, squalene synthesis inhibitors and squalene epoxidase inhibitors.

8. The use as claimed in claim 7, wherein the cholesterol biosynthesis inhibitor is selected from the group consisting of lovastatin,

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pravastatin, fluvastatin, simvastatin, CI-981, L-659,699, squalestatin 1 and NB-598.

9. The use as claimed in any of claims 1 to 5, wherein the cholesterol biosynthesis inhibitor is as specified in claim 7 or claim 8.

10. The use as claimed in any one of claims 6 to 8 wherein the β -lactam cholesterol absorption inhibitor is as defined in any one of claims 2 to 5.

10 11. A pharmaceutical composition for the treatment or prevention of atherosclerosis, or for the reduction of plasma cholesterol levels, comprising a β -lactam cholesterol absorption inhibitor, a cholesterol biosynthesis inhibitor and a pharmaceutically acceptable carrier.

15 12. A pharmaceutical composition as claimed in claim 11, wherein the cholesterol biosynthesis inhibitor is as defined in claim 7 or claim 8.

20 13. A pharmaceutical composition as claimed in claim 11 or claim 12, wherein the β -lactam cholesterol absorption inhibitor is as defined in any one of claims 2 to 5.

25 14. A method for preparing a pharmaceutical composition as claimed in any one of claims 11 to 13 comprising admixing a cholesterol biosynthesis inhibitor and a β -lactam cholesterol absorption inhibitor with a pharmaceutically acceptable carrier.

30 15. A method as claimed in claim 14 comprising admixing a cholesterol biosynthesis inhibitor as defined in claim 7 or claim 8, and a β -lactam cholesterol absorption inhibitor as defined in any one of claims 2 to 5 with a pharmaceutically acceptable carrier.

35 16. A kit comprising in separate containers in a single package pharmaceutical compositions for use in combination to treat or prevent atherosclerosis or to reduce plasma cholesterol levels which comprises in one container an effective amount of a cholesterol biosynthesis inhibitor in a pharmaceutically acceptable carrier, and in a second container, an

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effective amount of a β -lactam cholesterol absorption inhibitor in a pharmaceutically acceptable carrier.

17. A kit as claimed in claim 16 which comprises in one container an effective amount of a cholesterol biosynthesis inhibitor as defined in claim 7 or claim 8 in a pharmaceutically acceptable carrier, and in a second container, an effective amount of a β -lactam cholesterol absorption inhibitor as described in any one of claims 2 to 5 in a pharmaceutically acceptable carrier.
- 10 18. A method of treating or preventing atherosclerosis or reducing plasma cholesterol levels comprising simultaneously or sequentially administering to a mammal in need of such treatment an effective amount of a cholesterol biosynthesis inhibitor and a β -lactam cholesterol absorption inhibitor.
- 15 19. A method as claimed in claim 18, wherein the cholesterol biosynthesis inhibitor is as defined in claim 7 or claim 8.
- 20 20. A method as claimed in claim 16 or 17, wherein the β -lactam cholesterol absorption inhibitor is as defined in any one of claims 2 to 5.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/12291

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/395 // (A61K31/395, 31:365)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	WO, A, 93 02048 (SCHERING CORPORATION) 4 February 1993 cited in the application see page 27 - page 29, paragraph 2 see page 100 - page 101; claims ---	1-20
Y	STN INTERNATIONAL, KARLSRUHE FILE MEDLINE. AN=89338079. D. R. IILINGWORTH, 'An overview of lipid-lowering drugs.' see abstract & DRUGS, (1988) 36 SUPPL 3 63-71 -----	1-20



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search 28 April 1994	Date of mailing of the international search report 11.05.94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Orviz Diaz, P

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/12291

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 18-20 are directed to a method of treatment of the human body, the search has been carried out. It was based on the alleged effects of the compositions.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The expressions "beta-lactam cholesterol absorption inhibitor" and "cholesterol biosynthesis inhibitor" are not sufficient to characterize specific chemical compounds. The search had to be limited to the general concepts, to the formula in claim 2 and, ... (see annex for further information)
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

BOX I.2:

.... more particularly, to the specific compounds mentioned in the claims and in the pharmacological examples (see PCT, art. 6; Guidelines for Examination in the E.P.O., Part B, Chapter II.7, last sentence and Chapter III.3.7).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US 93/12291

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9302048	04-02-93	AU-A-	2398092	23-02-93
		CA-A-	2114007	04-02-93
		CN-A-	1069024	17-02-93
		EP-A-	0524595	27-01-93